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An Overview of the Clinical and Molecular Genetics of Wilms' Tumor

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INTRODUCTION

The treatment of Wilms' tumor is one of the remarkable success stories in pediatric oncology. Most children with Wilms' tumor in North America are registered on and subsequently treated according to National Wilms' Tumor Study Group (NWTSG) trials. In the past two decades, the NWTSG has contributed significantly to the development of therapeutic strategies for the management of these children. As a result, the majority of children with Wilms' tumor diagnosed in the 1990s are expected to survive and reach adulthood.

With a median age of 3.5 years at diagnosis, Wilms' tumor typically affects young children. However, the median age of diagnosis for certain children (those with bilateral Wilms' tumor, genitourinary anomalies, aniridia, or intralobar nephrogenic rests) is significantly younger, suggesting a predisposition for developing Wilms' tumor. Moreover, the associations of several genetically determined congenital anomalies (such as aniridia and Beckwith-Wiedemann syndrome [BWS]) with Wilms' tumor have also been of great help in identifying hereditary forms of Wilms' tumor and in determining possible locations of genes involved in its etiology.

A genetic model for the development of Wilms' tumor was originally proposed by Knudson and Strong in 1972 [1]. Based on analyses of age at diagnosis, and assuming constant mutation rates, they suggested that the development of Wilms' tumor required two rate-limiting genetic events. Children with genetic susceptibility would harbor an initial lesion in their germline, either inherited from a parent or resulting from a de novo mutation. The likelihood of a second genetic "hit" leading to tumorigenesis is thus greatly enhanced, compared with sporadic cases in which two rare independent somatic mutations are required. Subsequent genetic studies in a number of tumors have confirmed the Knudson model, demonstrating that the two postulated "genetic hits" constitute the inactivation of both alleles of a tumor suppressor gene. In many cases, the first allele is inactivated by a mutation within the gene itself, while the second allele is inactivated by a gross loss of chromosomal material, detected by so-called loss of heterozygosity (LOH) at one or more nearby polymorphic markers.

The two-event hypothesis predicts that susceptible individuals, such as familial cases, those with multifocal disease, and those with certain predisposing congenital anomalies, have a lower median age at diagnosis than those without these characteristics. Studies by NWTSG investigators have confirmed the expected younger average age at diagnosis in children with multifocal or bilateral Wilms' tumor, although not for those with familial Wilms' tumor [2], suggesting that susceptibility to Wilms' tumor is complex and possibly involves more than one genetic locus and interactions between genetic and epigenetic factors.

It is now clear that several chromosomal loci are involved in the etiology of this childhood malignancy. These include one at chromosome 11p13 (*WT1*), one at chromosome 11p15 (*WT2*), possibly one at Xq25-27, and a yet unidentified "familial" locus. In addition, several loci are involved in Wilms' tumor progression, including one at chromosome 16q, one at chromosome 1p, and *p53* at chromosome 17p13.

CHROMOSOME 11p13 AND *WT1*

The description of children with the rare congenital WAGR syndrome, consisting of malformations including *a*niridia, *g*enitourinary malformations, mental *r*etardation, and *W*ilms' tumor [3] who had constitutional deletions of band p13 of chromosome 11 [4], was the first clue to the genomic location of a Wilms' tumor gene. The fact that this first event involved apparent chromosomal loss also suggested that the underlying gene might be a tumor suppressor, rather than an oncogene. The subsequent observation of tumor-specific LOH for 11p13 DNA markers in some sporadic tumors supported the two-hit hypothesis and further suggested that sporadic tumors involved the same locus as the WAGR cases [5–8].

This Wilms' tumor gene, *WT1*, has now been cloned [9–11] and shown to be a developmentally regulated [12] transcription factor of the zinc finger family [13] with expression restricted to the genitourinary system, spleen, dorsal mesentery of the intestines, muscles, central ner-

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vous system, and mesothelium [14]. *WT1* is constitutionally deleted in all WAGR syndrome patients and there are examples of both bilateral [15] and sporadic [16] tumors with loss or independent mutations of the remaining allele, as predicted by the two-event tumor-suppressor model. Analyses in several series of sporadic Wilms' tumors, however, have found evidence for *WT1* mutation in only 5–10% of cases [17–19]. Thus, although *WT1* is undoubtedly a tumor-suppressor gene, it would appear to account for only a minority of Wilms' tumors.

Predisposition to Wilms' tumor is also a dominant feature of the rare Denys-Drash syndrome (DDS), which further consists of severe genitourinary anomalies (pseudohermaphrodism in males) and nephropathy progressing to renal failure (reviewed in [20]). Analysis of *WT1* in these patients has revealed the presence of constitutional, usually missense mutations, most frequently in the zinc finger region [21,22].

The majority of mutations described in Wilms' tumors are recessive, nonsense, or frame-shift mutations, whereas the majority of mutations described in DDS patients are dominant missense mutations [23]. All of the missense mutations described to date in DDS affect the DNA-binding ability of the predicted protein product and result in a far more severe phenotype than the one following complete constitutional inactivation of the *WT1* gene, i.e., WAGR syndrome.

CHROMOSOME 11p15 AND *WT2*

Wilms' tumor also occurs with increased frequency in BWS, a congenital and sometimes familial overgrowth and malformation condition variably consisting of macrosomia or hemihypertrophy, macroglossia, omphalocele, abdominal organomegaly, and ear pits or creases [24–26]. The BWS locus has been mapped to chromosome 11p15 based on consitutional karyotype abnormalities in rare patients [27], genetic linkage studies of families [28,29], and the rare occurrence of uniparental paternal isodisomy for 11p15 (the inheritance of two copies of one of the father's chromosomes 11 but neither of the mother's) [30,31].

In addition to the clinical association of Wilms' tumor and BWS, some sporadic Wilms' tumors have been identified with LOH restricted to markers at 11p15 not including 11p13 and *WT1* [28,32]. Intriguingly, an almost absolute bias to loss of the maternal allele has been found in Wilms' tumors [33], suggesting that *WT2,* the putative Wilms' tumor suppressor gene at 11p15, is subject to genomic imprinting [34]. That is, the gene is differentially marked, dependent on the parent of origin, such that the two alleles are not functionally equivalent [35]. Similarly, familial BWS segregates in a sex-specific fashion [28,36] and all reported cases of uniparental isodisomy have involved the paternal chromosome [30,31,37]. It is not yet known, however, whether the BWS locus and *WT2* are the same or at contiguous loci. Evidence from in vitro growth suppression experiments does, however, suggest the existence of a tumor suppressor locus (loci) distinct from the BWS locus [38].

Two *WT2* candidate genes, *IGFII* and *H19,* have been studied. Both are imprinted in normal humans [39], although in the opposite direction. A possibly substantial fraction of Wilms' tumors (without LOH at the DNA level) has been found to have altered imprints with resultant overexpression of the growth factor IGFII [39,40] and loss of expression of the tumor-suppressor *H19* [39–42]. This has raised the possibility that only one genetic event might be required to effectively alter (enhance or inactivate) the relevant loci.

Besides BWS, Wilms' tumor also occurs with increased frequency in a very similar overgrowth syndrome, Simpson-Golabi-Behmel (SGB), which is transmitted in a sex-linked fashion and is linked to Xq25-27 [43,44]. The SGB locus has not been cloned and therefore has not been evaluated in sporadic tumors. It has been speculated that if the BWS locus represents an imprinted locus, that the SGB locus might represent the imprintor locus [44].

FAMILIAL WILMS' TUMOR

Familial Wilms' tumor, although rare, appears to segregate in an autosomal dominant fashion, albeit with low and possibly variable penetrance. The low incidence has complicated the search for a gene involved in familial Wilms' tumor. However, linkage analyses in three large families have excluded both 11p13 and 11p15 (i.e., *WT1* and *WT2*) from conferring susceptibility to Wilms' tumor [45,46]. A third locus, at 16q (see below), has also been excluded as the familial Wilms' tumor gene [47]. Consequently the location of the familial locus (loci) remains unknown.

WILMS' TUMOR PROGRESSION GENES

In addition to the three genetic loci discussed above (*WT1, WT2,* familial), which are implicated in the predisposition or genesis of Wilms' tumor, there is evidence for genetic loci which may be involved in the progression to more malignant or aggressive tumors. LOH at chromosome 16q has been observed in ~15–20% of Wilms' tumors [48–50], and at chromosome 1p in ~10% [50]. Constitutional alterations at these genomic locations have not been associated with a predisposition to Wilms' tumor, but tumor-specific loss of either region has been associated with an adverse outcome [50].

The tumor-suppressor gene, *p53,* also appears to play a role in a subset of tumors [51]. Tumor-specific mutations appear to be specifically associated with the so-called anaplastic histology, a subgroup with a particularly ad-

verse prognosis. Indeed, when individual foci of anaplastic cells have been dissected from the bulk of the tumor, the mutations have been confined to the anaplastic foci demonstrating that these genetic events take place within the already developed tumor [52].

However, *p53* mutations have also been documented in occasional favorable histology Wilms' tumors [53]. Nevertheless, the high correlation between *p53* mutations and anaplastic Wilms' tumors suggest that *p53* alterations are required for progression to the anaplastic phenotype, albeit with possible additional events.

Apart from *p53,* which is associated with cell cycle regulation and apoptosis, the nature of the other putative Wilms' progression genes is unknown.

CONCLUSIONS

The last few years have provided dramatic breakthroughs in understanding the genetic factors involved in Wilms' tumorigenesis and hence in normal kidney development. The implications of these findings for the clinical management of children with Wilms' tumor are only now becoming apparent.

Identification of the genetic defects involved in Wilms' tumor will allow more precise genetic counseling in the future. Children who present with sporadic aniridia and are known to be at risk of developing Wilms' tumor can now undergo molecular analysis of germline DNA. This analysis should readily distinguish children with a mutation restricted to the aniridia gene, *Pax6,* from those with a chromosomal deletion encompassing the neighboring *WT1* gene. Similary, identification of the Wilms' tumor gene(s) residing at chromosome 11p15 and its relationshp with the BWS gene may allow genetic counseling for children with hemihypertrophy, whose increased risk of developing embryonal malignancies is now poorly defined.

Faced with an unknown risk of metachronous bilateral Wilms' tumor, identification of children with germline mutations in the different Wilms' tumor genes may distinguish those at risk from the majority of sporadic unilateral cases.

Finally, once the genetic factors that underlie Wilms' tumorigenesis have been identified, analysis of parental exposures associated with the development of this tumor may prove of great value to evaluate the mechanisms that lead to the described genetic defects particularly with reference to genomic imprinting.

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